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Full Length Research Paper

Molecular genetic analysis of male alternative strategy and reproductive success in the polygynous mating bat *Cynopterus sphinx*

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Cynopterus sphinx **is known to use polygynous mating system based on availability of resources, called resource defense polygyny. It is the primary mating strategy adopted by *C. sphinx*. In addition to such harem groups, a number of single adult males roost solitarily, nearer to the harems. Identifying the reasons behind the solitary roosting behaviour of such adult males is essential to further understand further the details of mating strategy in *C. sphinx*. In this context incomplete monopolization of harem females by harem males and nonharem male's access to harem females is to be observed. The role of nonharem males as probable fathers has not been tested. In the present study, PCR based RAPD markers were used to assess the paternity of harem males and nearby nonharem males to the young born in the harems. A total of 30 arbitrary primers were used to assign the parentage of offsprings. Samples from a total of 651 individuals (41 harem males, 295 females, 267 suckling pups and 48 solitary males) from 41 harems (dry season 14 harems and wet season 27 harems) of *C. sphinx* were tested for their RAPD-PCR patterns. The molecular results suggest that the nonharem males also gain access to harem females and sire more offspring in July-August breeding season (wet) than March-April breeding season (dry). These results suggest that nonharem males are reproductively active and enjoy some reproductive success.**

Key words: *Cynopterus sphinx*, nonharem male, alternative strategy, mating system, paternity assessment, RAPD markers.

INTRODUCTION

Studies on mating strategies have been one of the core aspects of behavioural ecology (Alcock, 2001). Understanding the evolutionary causes and consequences of social organization in a species requires an in depth knowledge of the mating system. In mammals, reproductive behaviour of females can be determined by observation of parturition and maternal care, which is a good indicator of motherhood. Therefore, a female's reproductive success is often determined by behavioural observations. In contrast, a male's reproductive success is much more difficult to determine. It has been reported that even detailed observations on male mating success may resulted in inaccurate estimates of reproductive success (Pemberton et al., 1992) and complex social systems with many competing males challenge the quantification of mating behaviour through observations. This is true for small-bodied, nocturnal and highly mobile animals like bats, wherein observations of behavior are rather difficult. Bats are of particular interest in sociobiology because of their peculiar life history. They form the second largest mammalian order, representing about a quarter of all mammals (Nowak, 1994). Interestingly, most species are social despite enormous ecological differences among them (Bradbury, 1977; Kunz, 1982).

In bats, most known mating associations are composed of a single male and several females (Kleiman, 1977; McCracken and Wilkinson, 2000). Such groups are usually called harems, although female composition is often unstable or only temporarily stable (Storz et al., 2000b; Dechmann et al., 2005). This has been observed in other polygynous mammals such as ungulates

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(Ruckstuhl and Neuhaus, 2000). In some cases monopolization of paternity by the dominant males are incomplete due to alternative strategies performed by satellite males to gain copulation. These alternative strategies include coalitions, forced copulations, or sperm competition (Clutton-Brock et al., 1979). Such parameters are frequently difficult to identify in wild populations by direct observation alone as mating system may be difficult to observe because of the nocturnal activity and the high mobility exhibited in this taxon.

As a result, the use of genetic techniques to accurately determine kinship in wild populations is increasingly common. One taxonomic group, which particularly benefits from the use of such techniques is the bats (Rossiter et al., 2000). Wide array of molecular markers are available to study the genetic variation within and among populations, to establish the phylogenetic relationship, identification of a taxon, genetic mapping and paternity assessment (Avise, 1994). The most common DNA fingerprinting strategy currently used for genetic analyses of natural populations is PCR based Random Amplified Polymorphic DNA (RAPD) analysis (Williams et al., 1990). RAPD is a DNA polymorphism assay based on the amplification of random DNA segments with single primers of arbitrary nucleotide sequence (Williams et al., 1990; Welsh and McClelland, 1990). It is widely used in the conservation, population and evolutionary biology because of its swiftness of results, cost-effectiveness and reproducibility (Williams et al., 1990; Hadrys et al., 1992).

Additionally, RAPDs are more cost-effective and less labour-intensive. The technique does not require a prior knowledge of DNA sequence information or the use of radioisotopes and generates DNA markers from much lesser tissue than needed for microsatellites. The results are directly visualized from the gels by screening the entire genome (Williams et al., 1993).

Recent studies on several bat species have employed molecular genetic methods to analyze the reproductive success within natural populations (Petri et al., 1997; Burland et al., 2001; Heckel et al., 1999, 2003; Ortega et al., 2003; Dechmann et al., 2005). However, knowledge about the mating systems in bats is far from understanding. The Indian short-nosed fruit bat, *Cynopterus sphinx*, belongs to the Old World fruit bats (Megachiroptera: Pteropodidae). It is a common plant-visiting bat, found throughout the Indo-Malayan region (Storz and Kunz, 1999). It weighs about 45 – 70 g. This bat roosts in the foliages either as solitarily or in 1 small group consisting of about 2 - 30 individuals (Balasingh et al., 1995; Bhat and Kunz, 1995; Storz et al., 2000a; Gopukumar et al., 2005; Karuppudurai et al., 2006, 2008). *C. sphinx* is a polygynous mating bat with polyestrous reproductive cycle having two well-defined and highly synchronous parturition periods per year (Krishna and Dominic, 1983).

C. sphinx is known to exhibit polygynous mating system (that is, prolonged association of one male with more than one female) based on resource availability and such behavior is popularly known as resource defence polygyny (Storz et al., 2000b). In *C. sphinx*, adult males are categorized into two groups, harem males and non-harem males. Harem males construct and defend tents (resource). Only those males who are in possession of a tent recruit females and gain mating access. This organization of bats is called harem. During breeding seasons these harem male bats defend critical resources to attract females, thereby facilitating a harem-polygynous mating system.

However, recent studies have shown that breeding population also consists of non-harem males and most of the time they occupy roosts that are adjacent to the harems (Storz et al., 2000b; Gopukumar et al., 2005; Karuppudurai et al., 2006, 2008). However, the role of non-harem males as probable fathers has not been examined in detail. Therefore, in the present study a PCR based RAPD strategy was used to study the paternity of harem males and nearby nonharem males to the young born in the harems.

MATERIALS AND METHODS

Study area

Fieldwork was conducted in Madurai (lat: 9° 58′ N; long: 78° 10′ E) and Palayamkottai (lat: 8° 44′ S; long: 77° 42′ E), Tamil Nadu, South India from January 2003 to December 2004 over a span of 2 years (4 breeding seasons). *C. sphinx* is known to construct tents from the leaves of several tree species found in the habitat. Within the study area, *Polyalthia longifolia* (mast tree) and *Borassus flabellifer* (palm tree) trees served as potential foliage-roosting sites for *C. sphinx* (Gopukumar et al., 1999; Balasingh et al., 1993, 1995). The breeding population of *C. sphinx* is subdivided into diurnal roosting colonies called “harems” consisting of a single male and one or more females and often one or more satellite males in adjacent trees in the study area.

Sample collection

Bats were collected from the foliage tents of *P. longifolia* (mast tree) and *B. flabellifer* (palm tree) using a hoop net with an extensible aluminium pole. The entire tree was enveloped with a 6 x 9 m nylon mist net (Avinet-Dryden, New York, USA) to prevent bats from escaping. In this study, 41 complete harem groups were captured and 48 adjacent solitary males were trapped in their diurnal roosts and pups and adults were individually marked. Bats were sampled over a period of four weeks immediately following each of four annual parturition periods: March – April 2003 and 2004 (dry season) and July – August 2003 and 2004 (wet season).

Table 1. List of primers and their sequences used in the present study.

S/No	Primer code	Primer sequence 5'-3'
1	A01	CAGGCCCTTC
2	A02	TGCCGAGCTG
3	A03	AGTCAGCCAA
4	A04	AATCGGGCTG
5	A05	AGGGGTCTTG
6	A06	GGTCCCTGAC
7	A07	GAAACGGGTG
8	A08	GTGACGTAGG
9	A09	GGGTAACGCC
10	A10	GTGATCGCAG
11	SK1	GTGTCTCAGG
12	SK2	GTGGGCTGAC
13	SK3	GTCCATGCCA
14	SK4	ACATCGCCCA
15	SK5	GTGGTCCGCA
16	SK6	TCCCGCCTCA
17	SK7	AACGCGTCGG
18	SK8	AAGGGCGAGT
19	SK9	GGAAGCCAAC
20	SK10	GGCTTGGCCT
21	OPA1	GTTTCGCTCC
22	OPA2	AGTCAGCCAC
23	OPA3	CATCCCCCTG
24	OPA4	AATCGGGCTG
25	OPA5	TGCGCCCTTC
26	OPA6	TGCTCTGCCC
27	OPA7	GAAACGGGTG
28	OPA8	GTGACGTATG
29	OPA9	GGTGACGCAG
30	OPA10	GTGATCGCAG

The breakdown of collection was as follows: 2003 dry season, 6 harems (76 adult females and 72 pups); 2004 dry season, 8 harems (79 adult females and 70 pups) and 2003 wet season, 15 harems (79 adult females and 71 pups); 2004 wet season, 12 harems (61 adult females and 54 pups). Additionally, all males that defended territories within the study area were sampled over a two year period (2003 - 2004) that spanned the dates of conception of sampled pups.

Bats were sampled when nearly all females had given birth but pups had not weaned as yet. Most pups were two to three weeks old at the time of sampling and all were matched with known mothers. Blood and or wing membrane biopsy samples of harem males, solitary males, harem females and pups of *C. sphinx* were collected during the breeding seasons (March/April and July/August). A medical punch was used for the excision of tissue (4 mm^2) and care was taken to place it in an area between the blood vessels to avoid injury (wing membranes healed within 3 - 4 weeks). After each sampling, the punched hole and the punch were disinfected with 70% ethanol. No negative effects of this treatment on the health of the bats were observed. It should also be noted that the bats frequently have natural injuries of this type in their wing membranes. The collected blood samples were immediately mixed with anticoagulant ACD, transferred to microcentrifuge tubes and sealed with parafilm. The blood and tissue samples were stored in ice, transported to the lab and stored at -20°C until DNA extraction (Worthington Wilmer and Barratt, 1996; Karuppudurai et al., 2007). Bats were held in net cages and released at their roosts during the evening of the same day they were captured.

Genomic DNA isolation and primer screening

Genomic DNA was isolated from wing-membrane biopsy samples using standard proteinase K digestion and phenol: chloroform extraction method (Sambrook et al., 1989). The quality and quantity of extracted DNA were checked using 0.7% agarose gel electrophoresis and spectrophotometric measurement at A260 and A280 nm (Hitachi U-2000, Tokyo, Japan). Finally the DNA pellets were stored at 4°C until further analysis. Alternately, DNA extraction was also performed with the DNeasy Tissue kit (Qiagen), following the manufacturer's instructions.

In the present study, RAPD-PCR was performed by using three series of primers (Table 1) namely A (A-01-A-10), SK (SK1-SK10) and OPA (OPA1-OPA10) each comprising ten primers (Microsynth, Switzerland). PCR conditions were optimized by varying concentrations of template DNA, primer, MgCl2 and Taq DNA polymerase.

Initial screening was done with all 30 primers using DNA from four (2 colonies from wet season and 2 colonies from dry season) colonies. PCR-RAPD analysis was repeated at least three times and the primers producing prominent reproducible bands were used for the analysis of 41 colonies. "Colony" or "Harem" is a group of individuals that roosted together regularly, which consisted of single adult male, adult females (3 - 21 individuals) and their pups.

Polymerase chain reaction (PCR)

PCR was carried out in 20 µl reaction containing 100 ng of template DNA, 2 µl of 10X PCR buffer (100 mM Tris HCl, 500 mM KCl, 0.8% Nonidet P40) with 1.5 mM MgCl2, 2 µl of 2 mM dNTP mixture, 5 µl of 2 µM primer, 1U of Taq DNA polymerase and 10 µl of H2O. All DNA amplifications were performed using an Applied Biosystems GeneAmp 2700 PCR system, with following cycling conditions including initial denaturation at 94°C for 5 min, followed by 35 cycles of denaturation at 94°C for

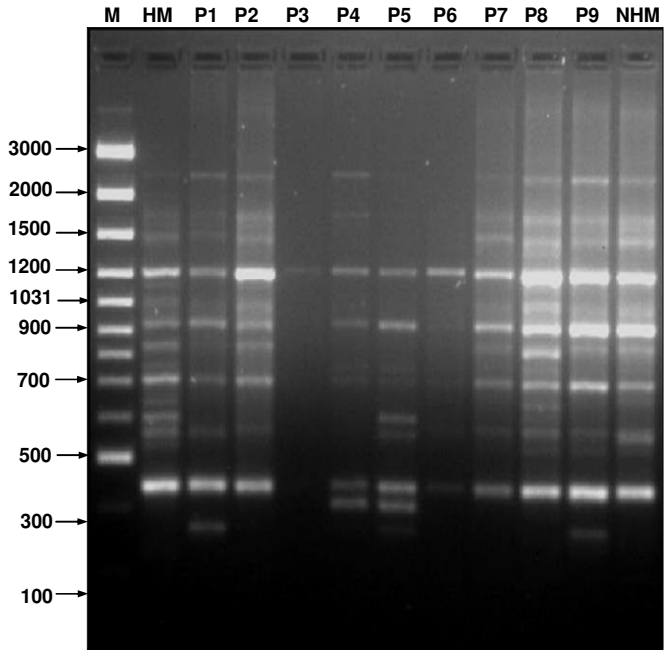

Figure 1. RAPD profile of *C. sphinx* (colony no: 1) obtained with primer A-05. Lane M, marker (100 bp DNA ladder); lane HM, harem male; lanes P1-P9, pups; lane NHM, nonharem male.

40 s, annealing at 30 - 36°C for 2 min (annealing temperature varying with the primers), extension at 72°C for 3 min and final extension at 72°C for 10 min. PCR products were electrophoresed on 2% agarose gel in 1x Tris-acetate-EDTA (TAE) buffer, stained with ethidium bromide (0.5 µg/ml) observed and photographed using gel documentation system (Biorad, USA, model 2000, Quantity One Software).

Data analysis

The RAPD data were analysed using NTSYS-pc version 2.0 (Numerical Taxonomy and Multivariate Analysis System) computer package (Rohlf, 1998). A genetic similarity (GS) between fathers and pups was computed based on Jaccard's coefficient of similarity as follows.

$$GS(ij) = a / (a + b + c)$$

Where:

GS (ij) is the measure of genetic similarity between individuals i and j.
a is the number of polymorphic bands that are shared by i and j.
b is the number of bands present in i and absent in j.
c is the number of bands present in j and absent in i.

Each RAPD fragment was treated as a unit character and was scored as 1 (present) or 0 (absent). The 1/0 matrix was prepared for all fragments scored and the data were used to generate Jaccard's similarity coefficients for RAPD bands (Jaccard, 1908). The Jaccard's coefficients were used to construct a dendrogram using the unweighted pair group method with arithmetic averages (UPGMA) or Weighted Average Linkage with the following formula.

$$dki = (np/n) \times dpi + (nq/n) \times dqi$$

where:

p, q = Indices indicating two clusters that are to be joined into a single cluster.
k = Index of the cluster formed by joining clusters p and q.
i = Index of any remaining clusters other than clusters p, q, or k.
np = Number of samples in the pth cluster.
nq = Number of clusters in the qth cluster.
n = Number of clusters in the kth cluster formed by joining the pth and qth cluster (n = np + nq).
dpq = Distance between cluster p and cluster q.

RESULTS

Over the course of two-year survey, 41 complete harem groups and 48 adjacent solitary males were trapped in their diurnal roosts. A total of 651 individuals (41 harem males, 295 females, 267 suckling pups and 48 solitary males) were sampled for the genetic analysis. Mother /pup pairs were sampled when pups were still attached to the teats of their mothers so that their relatedness was unambiguous. We tried to capture all females and their pups in a harem. In some capture attempts one or more females escaped and those pups were not included in our analysis.

In this study, only the harem males, pups and nonharem males were subjected to the paternity analysis. During the dry season 14 harem males, 142 offsprings and 18 nonharem males were captured and analyzed to assign the paternity of harem and nonharem males. The pairwise Jaccard's coefficients of genetic similarity matrix were generated for all the harem and nonharem males and offsprings. Based on the pairwise Jaccard's coefficients genetic similarity matrix, 132 of 142 offsprings were sired by harem males (average 94%) and the nonharem males sired only 10 offsprings (average 6%). A higher proportion of pups were sired by harem males in the March - April (dry) breeding season. Representative RAPD patterns generated by dry season colony 1 and their RAPD gel picture, pairwise Jaccard's coefficients genetic similarity matrix and dendrogram (UPGMA) are shown in Figure 1, Table 2 and Figure 2 respectively.

For example, the colony number 1 comprised a harem male, 9 females and 9 offsprings during the capture, among the 9 offsprings the harem male sired 7 offsprings

Table 2. Similarity matrix for Jaccard's coefficients for colony no: 1.

	HM	P1	P2	P3	P4	P5	P6	P7	P8	P9	NHM
HM	100.0	49.8	72.6	59.2	73.5	46.5	35.9	67.6	49.1	50.4	46.2
P1	49.8	100.0	58.2	31.8	49.4	74.3	63.0	48.5	31.7	41.5	38.4
P2	72.6	58.2	100.0	66.8	81.0	45.8	45.2	81.5	33.6	39.8	39.3
P3	59.2	31.8	66.8	100.0	60.4	25.6	29.7	64.1	31.9	22.3	35.0
P4	73.5	49.4	81.0	60.4	100.0	52.6	40.1	75.1	40.8	38.3	41.8
P5	46.5	74.3	45.8	25.6	52.6	100.0	52.0	45.1	38.3	40.3	40.1
P6	35.9	63.0	45.2	29.7	40.1	52.0	100.0	34.4	26.4	25.9	28.7
P7	67.6	48.5	81.5	64.1	75.1	45.1	34.4	100.0	33.4	40.9	36.0
P8	49.1	31.7	33.6	31.9	40.8	38.3	26.4	33.4	100.0	44.0	69.9
P9	50.4	41.5	39.8	22.3	38.3	40.3	25.9	40.9	44.0	100.0	47.1
NHM	46.2	38.4	39.3	35.0	41.8	40.1	28.7	36.0	69.9	47.1	100.0

Table 3. Similarity matrix for Jaccard's coefficients for colony no: 15.

	HM	P1	P2	P3	P4	NHM
HM	100.0	68.2	47.3	66.6	42.6	40.5
P1	68.2	100.0	51.8	89.1	49.5	42.1
P2	47.3	51.8	100.0	48.0	36.3	30.0
P3	66.3	89.1	48.0	100.0	44.9	36.6
P4	42.6	49.5	36.3	44.9	100.0	91.5
NHM	40.5	42.1	30.0	36.6	91.5	100.0

and the adjacent nonharem male sired only 2 offsprings (Figures 1, 2 and Table 2). The same way, we have analysed all the 14 harems captured during the dry season (March - April).

In the wet season 27 harem males, 125 offsprings and 30 nonharem males were captured and analyzed to assign the reproductive success of harem and nonharem males. Of the 125 offsprings the harem males sired only 52 offsprings (average 42%) and the nonharem males sired the rest 73 offsprings (average 58%). In the July - August (wet) breeding season the nonharem males sired a higher proportion of pups compared to harem males. Representative RAPD patterns generated by wet season colony 15 and their RAPD gel picture, pairwise Jaccard's coefficients genetic similarity matrix and dendrogram (UPGMA) are shown in Figure 3, Table 3 and Figure 4 respectively. The same way, we have analysed all the 27 harems captured during the wet season (July - August). The cumulative offsprings sired by harem and nonharem males from the 27 colonies are presented in the Table 4.

Over the course of 2 years (four breeding seasons) the nonharem males sire more offspring (58%) in July - August breeding season (wet) than March - April breeding season (dry) (6%) and the harem males sire more offspring (94%) in March - April breeding season (dry) than July - August breeding season (wet) (42%) (Table 4).

DISCUSSION

In the present study, the paternity assignments based on 30 RAPD random primers, revealed an unequal distribution of reproduction between harem and nonharem males. The results indicated that the *C. sphinx* study population is characterized by an extremely high within-season variance in male mating success, as expected from the harem-forming mode of social structure (Storz et al., 2000a, b). The monopolization of paternity by the harem males is incomplete due to alternative strategies used by satellite males to gain access to harem females and obtain some reproductive success.

During the dry season in our study area the average harem size was slightly higher compared to wet season because the dispersion of female *C. sphinx* is highly clumped due to limited roosting sites and the harem male sires 94% offsprings conceived during this period. However, during the wet season more roost sites are available and the harem size decreased because the females are widely dispersed as a result the harem males sire only 42% of offsprings, while nonharem males sire the rest of 58%.

Similar results have been reported in this species by Storz et al. (2000b, 2001). Apart from the mating success of nonharem males, low paternity for harem males can also occur as a result of female choice. Heckel et al. (1999)

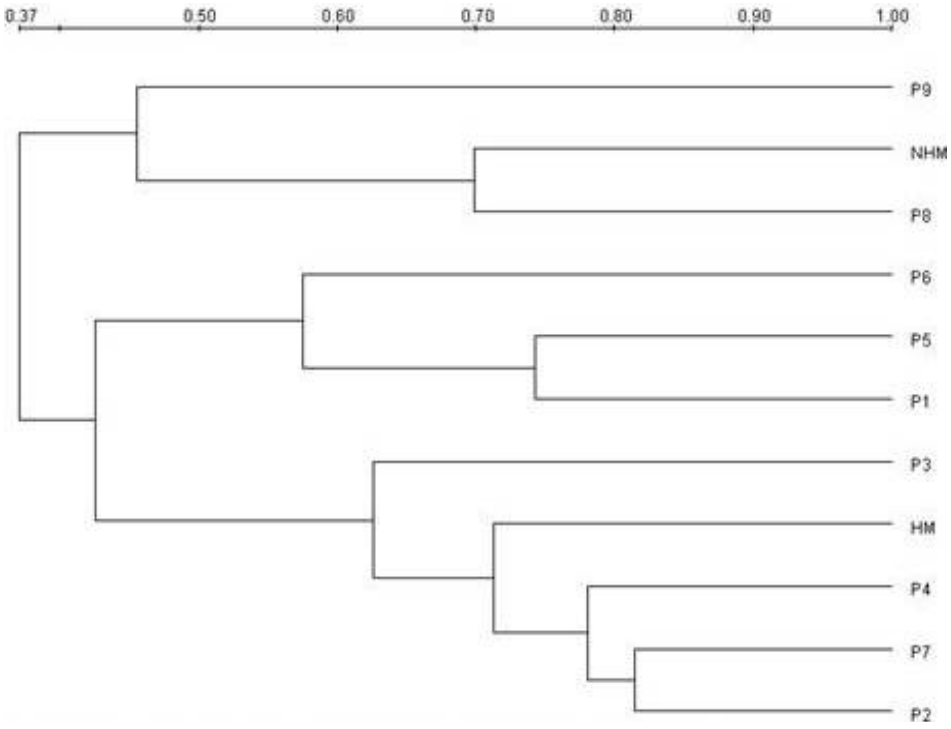

Figure 2. Dendrogram of genetic relationships among harem male, nonharem male and pups identified by RAPD analysis using UPGMA for colony no: 1.

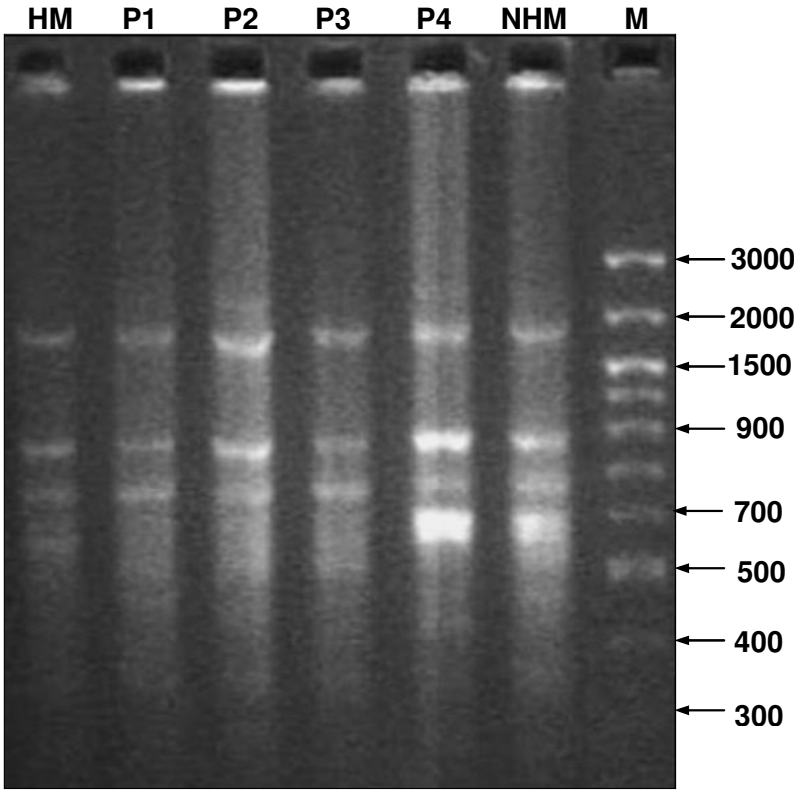

Figure 3. RAPD profile of *C. sphinx* (colony no: 15) obtained with primer SK7. Lane HM, harem male; lanes P1-P4, pups; lane NHM, nonharem male; lane M, marker (100 bp DNA ladder).

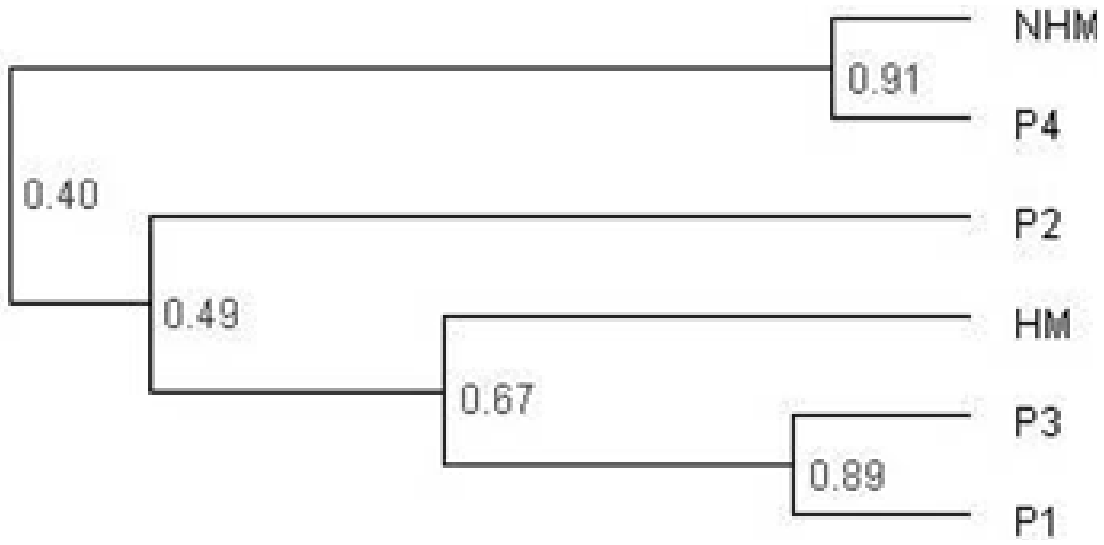

Figure 4. Dendrogram of genetic relationships among harem male, nonharem male and pups identified by RAPD analysis using UPGMA for colony no: 15.

reported the importance of female choice especially in highly mobile animals with harem system. It appears that female *Saccopteryx bilineata* actively select their roosting location and are highly mobile; some females shift roosting territories during the course of a day and some disperse to other colonies. Our recent radio-telemetry studies lend support to the observation of Heckel et al. (1999). We observed three postpartum estrus females (*C. sphinx*) visit a nonharem male exclusively during the night hours and engage in mating.

Balasingh et al. (1995) reported fluctuations in the harem size on a day-to-day basis, indicating that females periodically shifted their tents. Similarly, among the polygynous bats Artibeus jamaicensis (Ortega and Arita, 1999; Ortega et al., 2003), Phyllostomus hastatus (McCracken and Bradbury, 1977), Desmodus rotundus (Wilkinson, 1985) and *S. bilineata* (Heckel et al., 1999; Heckel and von Helversen, 2002), incomplete monopolization of females by harem males has been observed. The incomplete control of harem males over harem females increases the chances for nonharem males to fertilize some of the females.

The mating system most commonly described in bat species has been polygyny. However, a recent comprehensive review of bat mating systems has recognized the wide diversity of mating behaviour and has emphasized the importance of alternative strategy by males and multiple mating by females (McCracken and Wilkinson, 2000). This is supported by several genetic analyses which have shown that paternity is biased in polygynous mating systems. In harem groups of *S. bilineata*, it was demonstrated that 71% of offsprings born into a harem are not sired by the resident harem male, but are instead fathered by a number of different males, either adjacent harem males or peripheral males. However, harem males do gain greater overall reproductive success than peripheral males, as they achieve fertilization success both in their own and in other harems (Heckel et al., 1999; Heckel and von Helversen, 2002, 2003).

The dominant males in other polygynous species studied achieved slightly higher reproductive success than S. bilineata, although complete monopolization of females is rare. In the spear-nosed bat P. hastatus, the harem male fathers 60 - 90% of offsprings (McCracken and Bradbury, 1977, 1981), while the harem male in D. rotundus fathers approximately 45% of young (Wilkinson, 1985). In the later species, many different males, including those from other colonies contribute to the 55% offsprings. Similarly, the estimated paternity for dominant males of *A. jamaicensis* ranged from 33 - 90% followed by satellite (22%) and subordinate males (9%). Overall, most adult males belonging to a harem remained as dominant in the same group at least for two reproductive seasons (Ortega and Arita, 1999, 2000; Ortega et al., 2003). Comparable parentage studies on polygynous temperate-zone species have till date been restricted to some species such as the greater horseshoe bat *Rhinolophus ferrumequinum*. A study of one maternity colony demonstrated that no male fathered more than 12.5% of young in any cohort, despite the highly polygynous mating behaviour reported at mating sites in this species.

However, this finding may be at least partially explained by females from the same maternity colony visiting different mating sites (Rossiter et al., 2000). In those species for which less polygynous mating behaviour is predicted, genetic studies have generally confirmed low levels of male reproductive skew. The high number of different paternal alleles in the cohorts of young mouse-eared bats Myotis myotis and low proportion of paternal

Table 4. Summary of molecular data and number and percentage of pups sired by harem and nonharem males over a span of two years (four breeding seasons) between January 2003 and December 2004.

Breading season	Total no. harem males	Total no. of nonharem males	Total no. females	Total no. pups	Total no. of pups sired by		Total % of pups sired by	
					Harem males	Nonharem males	Harem males	Nonharem males
Dry (March - April)	14	18	155	142	132	10	94	6
Wet (July - August)	27	30	140	125	52	73	42	58
Total	41	48	295	267	184	83	68	32

half siblings identified among brown long-eared bats *Plecotus auritus* suggest that many males contribute to the gene pool in these species. Interestingly, the genetic data in both species also indicate that males do not generally achieve reproductive success within their own colony, suggesting that males and females from different maternity colonies mix during the mating season (Petri et al., 1997; Burland et al., 2001).

Taken together, the present results suggest that the nonharem males gain access to females and sire more offspring in July - August breeding season (wet) than March - April breeding season (dry). These results suggest that nonharem males are reproductively active, gain access to harem females and enjoy some reproductive success. Further investigations are needed to understand reproduction of nonharem males. The relatively high reproductive success of some nonharem males may indicate that solitary behaviour can be an acceptable alternative to territoriality. These solitary males had no costs for roost defense but sired number of juveniles. Reproduction by nonharem males is possible because harem males are not able to control the movement of the females in their harems (Balasingh et al., 1995; Heckel et al., 1999; Storz et al., 2000a, b). Females are able to choose their mating partners freely because harem males provide no paternal care. The social dominance of harem males exhibited by the persistent maintenance of a tent might indicate male quality and could therefore explain why most females reproduced with harem males. Investigations that follow the behaviour and reproductive success of individual males over their lifetime could clarify whether some nonharem males could potentially compensate lower reproductive success per year with longer persistence in the harem.

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